American Journal of
EPIDEMIOLOGY

Volume 154
Number 2
July 15, 2001

Sponsored by the Society for Epidemiologic Research
Published by Oxford University Press

HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEWS

GSTM1, *GSTT1*, and the Risk of Squamous Cell Carcinoma of the Head and Neck: A Mini-HuGE Review

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Squamous cell carcinoma of the head and neck (SCCHN) is a group of cancers of epithelial origin that may provide an ideal model for the study of gene-environment interaction. SCCHN includes squamous cell carcinomas of the oral cavity, pharynx, and larynx. Approximately 90% of the attributable risk for oral cancer and 80% of the attributable risk for larynx cancer results from tobacco use. Tobacco smoking has been demonstrated to increase the risk of SCCHN in a dose-response fashion. Polymorphisms of carcinogen-metabolizing enzymes, known to be involved in metabolism of carcinogens found in tobacco smoke, are relatively common in most populations. This paper provides a concise review of the 24 published studies that evaluated the risk of SCCHN in relation to two deletion polymorphisms of the glutathione *S*-transferase family: *GSTM1* and *GSTT1*. Patterns of risk based on the site of the tumor and on nationality are presented, as are some methodological weaknesses of the studies. The results of these studies are inconsistent, with some reporting weak-to-moderate associations and others finding no elevation in risk for the main effect of the gene. Few studies have directly evaluated the interaction with tobacco. Well-designed, population-based studies of adequate size are needed. *Am J Epidemiol* 2001;154:95–105.

glutathione transferase; *GSTM1*; *GSTT1*; head and neck neoplasms; neoplasms, squamous cell

GENE

The glutathione *S*-transferases (GSTs) are a family of enzymes known to play an important role in the detoxification of several carcinogens found in tobacco smoke (1). GSTs are dimeric proteins that catalyze conjugation reactions between glutathione and tobacco smoke substrates, such as aromatic heterocyclic radicals and epoxides. Conjugation facilitates excretion and thus constitutes a detoxification step. In addition to their role in phase II detoxification, GSTs also modulate the induction of other enzymes and proteins important for cellular functions, such as DNA repair (1). This class of enzymes is therefore important for maintaining cellular genomic integrity and, as a result, may play an important role in cancer susceptibility.

GST enzymes are coded for at five distinct loci, known as alpha, mu, theta, pi, and gamma. Two loci in particular, *GSTM1* and *GSTT1*, may be of relevance for susceptibility to squamous cell carcinoma of the head and neck (SCCHN). The *GSTM1* locus has been mapped on chromosome 1p13.3, while the *GSTT1* locus exists on chromosome 22q11.2. Persons with homozygous deletions of either the *GSTM1* or the *GSTT1* locus have no enzymatic functional activity of the respective enzyme. This has been confirmed by phenotype assays that have demonstrated 94 percent or greater concordance between phenotype and genotype (2–4). Deletion variants of *GSTM1* and *GSTT1* that result in no functional enzymatic activity for each locus have been characterized.

Received for publication November 16, 2000, and accepted for publication March 19, 2001.

Abbreviations: CI, confidence interval; GST, glutathione *S*-transferase; HPV, human papillomavirus; OR, odds ratio; PCR, polymerase chain reaction; SCCHN; squamous cell carcinoma of the head and neck.

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Three alleles have been identified at the *GSTM1* locus: one deletion allele and two others (*GSTM1a* and *GSTM1b*) that differ by C→G substitution at base position 534 (5, 6). This C→G substitution at base position 534 results in the substitution Lys→Asn at amino acid 172 (5). The Lys→Asn substitution results in no functional difference between the two alleles. As a result, *GSTM1a* and *GSTM1b* are categorized together as the positive conjugator phenotype. Two alleles have been identified at the *GSTT1* locus—one functional and the other nonfunctional (7). Persons who are of the homozygous deletion genotype are categorized into the negative conjugator phenotype, while those who carry either one or both of the functional alleles are grouped into the positive conjugator phenotype (5).

Two observations suggest a role for *GSTM1* or *GSTT1* genotypes and SCCHN susceptibility. First, exposure to tobacco smoke is the most important risk factor for SCCHN (8). Tobacco smoke is known to contain at least 55 carcinogens that can be grouped into three classes: polycyclic aromatic hydrocarbons, *N*-nitrosamines, and Asz-arenes (9, 10). Of the polycyclic aromatic hydrocarbons, benzo[*a*]pyrene-7,8-dihydrodiol-9,10-oxide (benzo[*a*]pyrene) is the most studied. Activation of benzo[*a*]pyrene results in its transformation into 7,8-diol-9,10-epoxide, a known substrate for the *GSTM1* enzyme (11).

Metabolism of carcinogens such as benzo[*a*]pyrene involves a balance of activation steps that produces reactive intermediates and detoxification steps that produce water-soluble, excretable compounds. Activation is often mediated by the cytochrome P-450s pathway and can result in the formation of compounds that can bind covalently to DNA, forming products known as adducts. Accumulation of DNA adducts at critical loci such as oncogenes or tumor suppressor genes can lead to somatic mutation and disruption of the cell cycle (10). Persons who do not have the ability to produce the *GSTM1* enzyme potentially accumulate more DNA adducts through their inefficiency at excreting activated carcinogens such as 7,8-diol-9,10-epoxide.

Other tobacco carcinogens, such as epoxybutanes and ethylene oxide, are known substrates for *GSTT1* (11, 12). *GSTT1*, like *GSTM1*, is known to play a role in phase II detoxification of carcinogens found in tobacco smoke as well as of other carcinogens found in pesticides, such as halomethanes and methyl bromide (1, 13). Unlike the *GSTM1* enzyme, however, *GSTT1* has both detoxification and activation roles (1, 14). For example, *GSTT1* is known to activate dihalomethanes to dichloromethane, which has been shown to cause liver and lung tumors in mice (1, 7).

Unlike any other member of the GST family, *GSTT1* is expressed not only in the adult liver but also in human erythrocytes and, as a result, is believed to play a more global role than *GSTM1* in detoxification of carcinogens in the body (14). This multifactorial role of the *GSTT1* enzyme is believed to reflect its heritage as the ancestral progenitor gene for all mammalian GST enzymes (1). The presence of *GSTT1* enzyme within red blood cells may allow red cells to act as a detoxification sink among those who are able to synthesize the enzyme (1). Interestingly, if the capacity for removal of detoxification products from the circulation is exceeded among those with *GSTT1* functionality, the risk of carcinogenesis may be increased compared with risk among those who have no function of the enzyme (1).

The second observation suggesting that *GSTM1* or *GSTT1* genotypes are important for SCCHN susceptibility is that GST enzymes are expressed in the squamous mucosa of the head and neck with some site specificity (15–17). For example, normal and malignant squamous cells of the larynx have been shown to express the GST-mu isoform in the highest concentration compared with GST alpha, pi, gamma, or theta (18–21). *GSTP1* is found in the greatest concentration in the oral and pharyngeal mucosa of the head and neck compared with the other GST enzymes (22–25).

GENE VARIANTS

An extensive review of gene variants for *GSTM1* and *GSTT1* has been published previously (5, 26) and will be updated only briefly here. Medline and PubMed were searched by using the keywords "glutathione *S*-transferases," "*GSTM1*," and "*GSTT1*." Reference lists from published articles were also reviewed. Papers written in English and published between 1993 and 2000 were reviewed.

The majority of the studies reviewed were case-control in design. Variation in frequencies reported among the same ethnic groups may be due to differences in study size and source of control group. Studies using hospital or other non-population controls may not represent the true genotype distribution for a given population.

In the United States, the reported range of the *GSTM1* deletion genotype varies by ethnic group. Reported frequencies from hospital-based case-control studies range from 23 to 41 percent for persons of African descent, from 32 to 53 percent for persons of Asian descent, from 40 to 53 percent for those of Hispanic descent, and from 35 to 62 percent for those of European descent (26, 27). Several population-based studies have reported prevalences ranging from 48 to 57 percent for the *GSTM1* deletion genotype among US Caucasians (28–31).

South American case-control (non-population-based) studies have reported frequencies of 21 percent for Chileans (32), 55 percent for Caucasian Brazilians, 33 percent for Black Brazilians, and 20 percent for Amazonian Brazilians (33).

European case-control studies have indicated variation in the frequency of the *GSTM1* deletion genotype. Among the French, 46 percent have been reported to carry the null genotype (34). A large cross-sectional study conducted among Italians reported a frequency of 53 percent (35), and studies conducted in Hungary and the Slovak Republic measured frequencies of 44 and 50 percent, respectively (36, 37).

Groups such as Pacific Islanders and Malasians have a reported *GSTM1* deletion genotype frequency of 62–100 percent. Other Asian populations, such as the Japanese and Chinese, also have a high frequency of *GSTM1* deletions. Reported frequencies range from 48 to 50 and 35 to 63 percent, respectively (5). A population-based study conducted among the Chinese reported a frequency of 51 percent for

the *GSTM1* deletion genotype (38). Two Korean case-control studies found frequencies of 53 and 56 percent, respectively, for the *GSTM1* deletion genotype (39, 40).

Studies of *GSTT1* null genotype demonstrate that, in the United States, deletion of *GSTT1* is less common than the *GSTM1* deletion genotype. Among those of European ancestry, 15–31 percent have no functional *GSTT1* enzyme. African Americans have frequencies ranging from 22 to 29 percent, while those of Hispanic origin carry *GSTT1* deletions of 10–12 percent (26, 27, 30, 31).

European studies have reported that the *GSTT1* deletion genotype was present among 21 percent of Italians and 28 percent of Slovakians (35, 37). One South American study found that 19 percent of both Caucasian and Black Brazilians had the deletion genotype compared with 11 percent of Amazonian Brazilians (33).

Asians have the highest reported *GSTT1* deletion genotype. One study reported that 58 percent of Chinese and 38 percent of Malaysians have the *GSTT1* null genotype (41); two case-control studies measured 42 and 46 percent among Koreans, respectively (39, 40). However, a recent population-based study conducted among the Chinese found a prevalence of 46 percent for the *GSTT1* deletion genotype among their study subjects (38).

DISEASE

SCCHN is a group of cancers defined by their anatomic location (oral cavity, pharynx, and larynx) and their common cell of origin (squamous cell). Roughly three times as many incident cases of oral cavity and pharynx cancer are diagnosed each year in the United States compared with incident cases of larynx cancer (42). For the year 2000, approximately 40,400 incident cases of SCCHN were diagnosed in the United States, and 20,400 deaths occurred from it (42).

Worldwide, it has been estimated that approximately 500,000 incident cases are diagnosed each year (43). Within the developing world, SCCHN represents the third most common cancer among men and the fourth most common among women (44). Five-year survival has remained unchanged during the past 5 decades: approximately 47 percent of patients with oral or pharyngeal squamous cell carcinoma and 44 percent of patients with laryngeal squamous cell carcinoma die 5 years after diagnosis (42).

Tobacco smoking is the strongest risk factor for SCCHN. Various population-based studies of male cigarette smokers have reported relative risks of 3–13 for ever-smokers (45). When the amount of tobacco smoked is examined, a dose-response trend is demonstrated. Relative risks, adjusted for alcohol use, of 1.6 (95 percent confidence interval (CI): 0.9, 2.7) for light smokers (<20 cigarettes per day for 20 or more years), 2.8 (95 percent CI: 1.8, 4.3) for moderate smokers (20–39 cigarettes per day for 20 or more years), and 4.4 (95 percent CI: 2.7, 7.2) for heavy smokers (≥40 cigarettes per day for 20 or more years) have been found (45). In spite of a lower incidence compared with men (roughly twice as many men as women are diagnosed with incident disease in the United States), it has been suggested that women have a relatively increased risk for SCCHN per tobacco smoke dose of carcinogens (46–52). Relative risks of 3.0 (95 percent CI: 1.9, 5.2) for light smokers, 4.4 (95 percent CI: 2.7, 7.2) for moderate smokers, and 10.2 (95 percent CI: 5.2, 20.4) for heavy smokers have been measured among women (45).

Alcohol consumption is also linked to an increased risk of SCCHN. For those men and women who consumed more than 30 drinks of alcohol per week, the risk of developing SCCHN was nine times that of a nondrinker (8). Among nonsmokers, odds ratios (OR) of 1.9 (95 percent CI: 0.4, 9.6), 2.3 (95 percent CI: 0.4, 12.4), and 9.1(95 percent CI: 1.7, 48.5) have been demonstrated for light, moderate, and heavy alcohol consumers, respectively, compared with abstainers (53).

Evidence of synergism is seen among persons who smoke tobacco and drink alcohol. Relative risks of approximately 40 have been found among those who smoke 40 or more cigarettes a day and consume 30 or more drinks per week (45). A recent case-control study conducted in Brazil among 784 cases of SCCHN measured an OR of 20 for those with the greatest cumulative measures of alcohol and tobacco (53). Blot et al. (8) have estimated that approximately 75 percent of the attributable risk of SCCHN results from the combined effects of tobacco and alcohol.

Human papillomavirus (HPV) may play a role in the etiology of SCCHN. More than 30 studies (most of which have been case-series) have been conducted examining the association between SCCHN and HPV genomic DNA (54). Use of different molecular methods in identification of HPV makes comparison of these studies difficult; however, three of the larger studies suggested an increase in risk for oral cancer among those infected with high-risk HPV types 16 and 18 (55–57). The overall estimates of high-risk HPV prevalence among persons with SCCHN vary from 8 to 100 percent, with an average prevalence of 35 percent when polymerase chain reaction (PCR) methods are used to detect the virus (58). The exact role of HPV in SCCHN etiology remains unclear.

Epidemiologic studies have demonstrated increased risk of SCCHN among the elderly (59, 60), African Americans (61, 62), patients of low socioeconomic status (63), and certain occupations (50, 64). Diets poor in fruits and vegetables have been identified as a risk factor for SCCHN (59, 65–68). Use of other tobacco products, such as chewing tobacco and snuff, have also been identified as risk factors for oral cavity and pharynx cancer, as has the use of alcohol-based products such as mouthwash (49, 52, 69–71).

ASSOCIATIONS

The search strategy used for identifying papers for review is the same as the strategy defined in the gene variant section. Additional search words included "head and neck cancer." Papers published from 1993 to 2000 and written in English were considered eligible. Studies of squamous cell carcinoma of the esophagus were excluded.

Twenty-four hospital-based case-control studies of *GSTM1* and *GSTT1* and risk of SCCHN have been published to date. Results of these studies are individually sum-

TABLE 1. Carcinogen-metabolizing enzymes and risk of squamous cell carcinoma for the Americas

Reference (no.)	Population	Site	No. of cases	No. of controls	Source of control group	Deletion genotype among controls (%)	Matching	Genotype	OR*	95% CI*	Gene-tobacco interaction		
											Genotype	OR	95% CI
Hamel et al. (87)	Canada	Head and neck	90	90	Hospital population	*GSTM1* (58) *GSTT1* (22)	Ethnicity, year of birth, gender	*GSTM1* *GSTT1*	0.96 2.6	0.5, 1.7 1.1, 5.9	*GSTT1*	6.5	2.3, 19.0
Trizna et al.(89)	US	Head and neck	186	42	Relatives Blood donors	*GSTM1* (48) *GSTT1* (36)	Age, race, gender	*GSTM1* *GSTT1*	2.4 1.5	1.2, 4.7 0.7, 3.0			
Park et al. (77)	US	Oral	135	135	Relatives and friends Hospital	*GSTM1* (51)	Age, race, site of recruitment, gender	*GSTM1*	1.0	0.6, 1.7	*GSTM1*	1.3	0.5, 3.4†
Cheng et al. (92)	US	Head and neck	162	315	Hospital Spouses	*GSTM1* (43) *GSTT1* (18)	Tobacco, age, gender, ethnicity	*GSTM1* *GSTT1*	1.5 2.3	1.0, 2.2‡ 1.4, 3.6			
Olshan et al. (85)	US	Head and neck	182	202	Hospital	Caucasians *GSTM1* (15) *GSTM1* (13) African Americans *GSTM1* (40) *GSTT1* (20)	Age, gender	*GSTM1* *GSTT1*	1.1 1.2	0.7, 1.7§ 0.7, 2.3	*GSTM1* *GSTT1*	 1.2 2.8 5.9 2.7 3.7 7.0	 0.3, 4.5¶ 0.9, 8.8 2.1, 17.0 0.5, 12.9# 0.7, 19.4 2.2, 22.0
Park et al. (39)	US	Oral	164	346	Hospital	Caucasians *GSTM1* (49) African Americans *GSTM1* (16)	Age, race, site of recruitment, gender	*African Americans* *GSTM1* *Caucasians* *GSTM1*	3.1 1.4	1.1, 8.5** 0.7, 2.8	*African Americans* *GSTM1* *Caucasians*	2.0 5.4 2.5 5.3 1.6	0.3, 14†† 1.2, 24 0.7, 9.2 0.4, 75 0.6, 4.4
McWilliams et al(86)	US	Head and neck	160	149	Recruited volunteers	*GSTM1* (47) *GSTT1* (18)	None	*GSTM1* *GSTT1*	1.0 0. 9	0.6, 1.6 0.5, 1.7			

* OR, odds ratio; CI, confidence interval.
† Among cases only and greater than 30 pack-years of smoking.
‡ Adjusted for gender, age, ethnicity, smoking status, and alcohol use.
§ Adjusted for age, gender, and race.
¶ Adjusted for age, race, gender, average number of drinks per week, *GSTMl* null genotype, and average number of cigarettes per day (0, 1–19, ≥20).
# Adjusted for age, race, gender, average number of drinks per week, *GSTTl* null genotype, and average number of cigarettes per day (0, 1–19, ≥20).
** Adjusted for tobacco use, alcohol consumption, and site of recruitment.
†† Adjusted for alcohol consumption, site of recruitment, *GSTM1* null genotype, and pack-years (nonsmoker, ≤24, ≥24).

TABLE 2. Carcinogen-metabolizing enzymes and risk of squamous cell carcinoma for Europe

Reference (no.)	Population	Site	No. of cases	No. of controls	Source of control group	Deletion genotype among controls (%)	Matching	Genotype	OR*	95% CI*	Gene-tobacco interaction		
											Genotype	OR	95% CI
Deakin et al. (75)	Britain	Oral	40	577	Hospital	*GSTM1* (35) *GSTT1* (19)	None	*GSTM1* *GSTT1*	1.0 0.6	0.5, 1.9 0.2, 1.7			
Worral et al. (81)	Britain	Oral	100	467	Hospital	Data not reported	None	*GSTM1* *GSTT1*	1.0 1.0				
Coutelle et al. (74)	France	Oral Larynx	21 18	37	Alcoholics	*GSTM1* phenotype	Only smokers included		*Oral*				
								GSTM1	1.8	0.5, 6.2			
									Larynx				
								GSTM1	4.7	1.0, 21.8			
Jourenkova et al. (91)	France	Larynx	129	172	Hospital	*GSTM1* (52) *GSTT1* (16)	Age, gender, site of recruitment Only smokers included	*GSTM1* *GSTT1*	1.6 1.4	1.0, 2.8† 0.7, 2.9			
Jourenkova-Mironova et al. (82)	France	Oral and pharynx	121	172	Hospital	*GSTM1* (52) *GSTT1* (16)	Age, gender, site of recruitment Only smokers included	*GSTM1* *GSTT1*	0.9 2.0	0.5, 1.5‡ 1.0, 4.0	*GSTM1* *GSTT1*	0.6 1.0 0.8 3.3	0.3, 1.4§ 0.4, 2.6 0.3, 2.6 1.3, 8.1
Jahnke et al. (88)	German	Larynx	269	216	Criteria not given	*GSTM1* (52) *GSTT1* (13)	None	*GSTM1* *GSTT1*	2.8 0.5	1.1, 6.4 0.2, 1.1			
Mathias et al. (79)	German	Head and neck	398	219	Hospital	*GSTM1* (53) *GSTT1* (22)	None		*Oral/pharynx*				
								GSTM1 *GSTT1*	1.2 1.5	0.8, 2.0¶ 0.9, 2.5			
									Larynx				
								GSTM1 *GSTT1*	1.0 0.9	0.7, 1.5 0.5, 1.4			
Ophuis et al. (80)	Netherlands	Head and neck	185	207	Blood donors	*GSTM1* (52) *GSTT1* (20)	None	*GSTM1* *GSTT1*	1.0 0.95	0.65, 1.43 0.6, 1.6			
Lafuente et al. (72)	Spain	Larynx	78	78	Hospital	*GSTM1* phenotype	Age, tobacco use Only smokers included	*GSTM1*	2.5	1.8, 3.1			
Lafuente et al. (23)	Spain	Larynx	160	158	Hospital	*GSTM1* phenotype	None Only smokers included	*GSTM1*	1.9	1.2, 3.1			
Gonzalez et al. (78)	Spain	Head and neck	75	200	Blood donors	*GSTM1* (54)	None	*GSTM1*	1.3	0.8, 2.3			

* OR, odds ratio; CI, confidence interval.
† Adjusted for age, gender, duration of smoking, smoking status, amount of tobacco smoked, and alcohol use.
‡ Adjusted for gender, age, daily consumption of tobacco, smoking status, and alcohol consumption.
§ Adjusted for gender, age, daily consumption of tobacco, smoking status, and alcohol consumption. Association for <30 years of smoking (referent) compared with ≥30 years of smoking.
¶ Adjusted for age and gender.

TABLE 3. Carcinogen-metabolizing enzymes and risk of squamous cell carcinoma for Asia

Reference (no.)	Population	Site	No. of cases	No. of controls	Source of control group	Deletion genotype among controls (%)	Matching	Genotype	OR*	95% CI*	Gene-tobacco interaction		
											Genotype	OR	95% CI
Kihara et al. (90)	Japan	Head and neck	158	474	Hospital	*GSTM1* (49)	None		*Nonlarynx*				
								GSTM1	1.9	0.8, 4.5†			
									Larynx				
								GSTM1	3.9	1.0, 17.7			
Sato et al. (94)	Japan	Oral	142	142	Hospital	*GSTM1* (45)	Age, gender	*GSTM1*	2.2	1.4, 3.6	*GSTM1*	3.1	1.6, 5.9‡
												3.9	1.6, 9.1
												16.2	4.3, 61.0
Tanimoto et al. (84)	Japan	Oral	100	100	Hospital	*GSTM1* (40)	Age, gender	*GSTM1*	1.0				
Katch et al. (93)	Japan	Oral	92	146	Hospital	*GSTM1* (46)	None	*GSTM1*	1.7	1.0, 2.8§	*GSTM1*	2.0	0.9, 4.6¶
						GSTT1 (51)		*GSTT1*	0.9	0.5, 1.5	*GSTT1*	0.9	0.4, 1.9
Morita et al. (83)	Japan	Head and neck	145	164	Hospital	*GSTM1* (61)	None	*GSTM1*	1.0				
Hung et al. (76)	Taiwan	Oral	41	123	Population	*GSTM1* (58)	Age, gender	*GSTM1*	1.0	0.5, 2.0#			
						GSTT1 (53)		*GSTT1*	1.2	0.6, 2.5			

* OR, odds ratio; CI, confidence interval.
† Adjusted for age.
‡ Adjusted for gender and age. Odds ratios calculated for increasing dose of tobacco.
§ Adjusted for age and gender.
¶ Adjusted for age and gender. Association for smokers versus nonsmokers (referent).
# Adjusted for age and ethnicity.

marized in tables 1–3. Two representative studies have been selected for more detailed discussion, one of which was conducted in the United States and the other of which was conducted in Germany. Trends of results based on tumor site (oral/pharynx and larynx) are presented, since GSTs can demonstrate site specificity, and there is heterogeneity in risk based on tumor site. Trends based on nationality will also be summarized, since the frequency of GST polymorphisms varies by ethnicity as well as by the species of tobacco grown and smoked (5, 9). A brief discussion of methodological weaknesses and how they may influence the validity of the trends will be given.

GSTM1 overview

The earliest published study of *GSTM1* and SCCHN of which we are aware focused on the enzyme phenotype. A study of laryngeal cancer reported an OR of 2.5 (95 percent CI: 1.8, 3.1) for persons lacking phenotypic expression of the *GSTM1* enzyme (72). Results published later by the same group reported an OR of 1.9 (95 percent CI: 1.2, 3.1) for *GSTM1* null phenotype and risk of larynx cancer (73). Coutelle et al. (74) reported an OR of 4.7 (95 percent CI: 1.0, 21.8) for larynx cancer among those who lacked *GSTM1* enzyme expression and an OR of 1.8 (95 percent CI: 0.5, 6.2) for cancer of the oral cavity or pharynx after adjustment for age.

Twenty-one published studies have examined the risk of SCCHN and the *GSTM1* deletion genotype. Thirteen have reported ORs of between 0.9 and 1.3 for the *GSTM1* deletion polymorphism (75–87), while the remaining eight have reported ORs of between 1.4 and 3.9 (88–95). Among the larger studies (those with 150 cases or more), five found ORs ranging from 1.4 to 3.9 (89, 90, 92, 95, 96), while four measured ORs ranging from 0.9 to 1.2 (79, 80, 85, 86).

Two representative studies. In 1999, Cheng et al. (92) published one of the largest US studies conducted to date. Cases were recruited from outpatients in the Department of Head and Neck Surgery at M. D. Anderson Cancer Center, Houston, Texas, between May 1995 and April 1998. Details of the sampling strategy for selection of cases were not given, and it was not stated whether the 162 cases recruited for the study represented incident or prevalent disease.

Controls were selected from two sources: outpatients at a health maintenance organization and spouses of outpatients at M. D. Anderson Cancer Center. Controls were matched to cases on the basis of tobacco exposure, age, gender, and ethnicity. Exposure data on tobacco and alcohol were collected by self-administered questionnaire. After adjustment for age, gender, ethnicity, tobacco, and alcohol, an OR of 1.5 (95 percent CI: 1.0, 2.2) for *GSTM1* was reported.

Another large study, conducted in Germany, was published in 1998. Matthias et al. (79) identified 398 cases of SCCHN diagnosed from 1994 to 1996 at the Department of Otorhinolaryngology, Virchow-Klinikum, Humboldt University, Berlin, Germany. The authors did not give information about whether the cases collected represented incident or prevalent disease. This case-control, hospital-based study collected almost all consecutively diagnosed cases of SCCHN at their institution. Data were also collected on those persons who refused to participate in the study, and this information was used to evaluate whether refusal to participate was correlated with the stage of the cancer.

Controls were selected from outpatients in the same department who were undergoing surgery for hearing loss or septumplasty. Tobacco and alcohol exposures were measured by interview. However, the investigators were not able to ascertain exposure data on 50 percent of the controls. These investigators found an OR of 1.2 (95 percent CI: 0.8, 2.0) for oral/pharynx cancer and an OR of 1.0 (95 percent CI: 0.7, 1.5) for larynx cancer after adjustment for age and gender.

Trends based on nationality. The largest German studies have suggested minimal increase in risk (ORs of 1.2 for both studies) after adjustment for age and gender (79, 88). Three of the four largest US studies have found ORs of 1.4–3.1 (89, 92, 95). Park et al. (95) reported a strong association for risk of oral cancer as modified by race. An OR of 3.1 (95 percent CI: 1.1, 8.5) for African Americans and an OR of 1.4 (95 percent CI: 0.7, 2.8) among Caucasians were reported after adjustment for tobacco use, alcohol consumption, and site of subject recruitment. Among Japanese studies, the largest has shown an increased risk for larynx (OR = 3.9) and oral/pharyngeal (OR = 1.9) cancers among smokers, after adjustment for age (90).

Trends based on site of tumor. Among those studies that have examined the risk of oral cavity squamous cell carcinoma, the majority have found no association with the *GSTM1* deletion genotype (75–77, 81, 82, 84). It is worth noting, however, that among the Japanese, the majority of oral cancer studies have found an association with ORs ranging from 1.7 to 2.2 (90, 93, 94). Katoh et al. (93) reported an OR of 1.7 (95 percent CI: 1.0, 2.8) for risk of oral cancer for those with the *GSTM1* deletion genotype after adjustment for age and gender. Sato et al. (94) calculated an unadjusted OR of 2.2 (95 percent CI: 1.4, 3.6) for risk of oral cancer among those with the *GSTM1* deletion genotype. The study by Kihara et al. (90) confirmed the results of the study by Katoh et al., with an age-adjusted OR of 1.9 (95 percent CI: 0.8, 4.5) among cases of oral/pharyngeal cancer.

Three studies have found an association with laryngeal squamous cell carcinoma and the *GSTM1* deletion genotype, with ORs ranging from 1.6 to 3.9 (88, 90, 91). Kihara et al. (90) reported that patients who smoked and carried the *GSTM1* deletion genotype were almost four times more likely to be diagnosed with squamous cell carcinoma of the larynx before age 60 years compared with controls. Among 129 cases of larynx cancer and 172 controls in France, an OR of 1.6 (95 percent CI: 1.0, 2.8) was detected after adjustment for age, gender, years of smoking, smoking status, daily consumption of tobacco, drinking status, and daily consumption of alcohol (91). Finally, the largest of the three studies, conducted in Germany with 269 patients and 216 controls, found an unadjusted OR of 2.8 (95 percent CI: 1.1, 6.4) (88).

GSTT1 overview

Fourteen studies have examined the *GSTT1* deletion genotype and risk of SCCHN. Six have suggested an increase in risk, with ORs ranging from 1.4 to 2.6 (79, 82,

87, 89, 91, 92). Other studies, however, have reported ORs of 0.5 to 1.2 (75, 76, 80, 81, 85, 86, 88, 93).

Two representative studies. Details of the studies by Cheng et al. (92) and Matthias et al. (79) were presented in the *GSTM1* overview section. Cheng et al. found an OR of 2.3 (95 percent CI: 1.4, 3.6) for those with the *GSTT1* deletion genotype after adjustment for age, gender, ethnicity, smoking status, and alcohol status. Matthias et al. demonstrated an OR of 1.5 (95 percent CI: 0.9, 2.5) for oral/pharyngeal cancer and an OR of 0.9 (95 percent CI: 0.5, 1.4) for larynx cancer after adjustment for age and gender.

Trends based on site of tumor. Of the studies that conducted a tumor site-specific analysis, two demonstrated an increased risk for oral squamous cell carcinoma and the *GSTT1* deletion genotype (OR = 2.0 and 1.5, respectively) (79, 82), while four did not (75, 76, 81, 93). Among the studies that did not find an association, it is worth noting that the largest contain only 100 cases, and two of the studies contained fewer than 50 cases.

For cancers of the larynx, two studies have reported conflicting results. Jahnke et al. (88) reported an unadjusted OR of 0.5 (95 percent CI: 0.2, 1.1) for those with deletion of the *GSTT1* gene, while Jourenkova et al. (91) reported an OR of 1.4 (95 percent CI: 0.7, 2.9) after adjustment for gender, age, duration of smoking (years), smoking status, daily consumption of tobacco (g/day), and drinking status.

Trends based on nationality. No obvious trends based on nationality have been noted for the *GSTT1* deletion genotype and risk of SCCHN.

Summary

In summary, the results of the studies reviewed are inconsistent, with some studies that reported weak-to-moderate associations and others that found no elevation in risk. Thus, the evidence for the role of *GSTM1* and *GSTT1* and the risk of SCCHN is inconclusive.

Methodological weaknesses of studies

A general methodological concern of the studies reviewed was the potential selection bias that may have been introduced by a poorly defined study base. Failure to properly sample from the base in a hospital case-control study can bias gene-environment interactions if the controls do not reflect the exposure and/or genotype distributions of the source population. Several of the studies reviewed used controls that were either persons with other diseases associated with the exposure or other persons, such as friends, spouses, or volunteers, who may have biased exposure distributions (77, 86, 87, 89, 92). Only one study reviewed used a population-based sampling frame (76).

Some studies used matching of controls to cases (72, 76, 77, 82, 84, 85, 87, 89, 92, 94, 95). Matching is often utilized to increase the efficiency of the statistical adjustment of confounding factors. However, selection bias and residual confounding may be introduced when matching factors are not accounted for in the analysis (97). Several studies reviewed did not adjust for matching factors (82, 84, 87, 95).

Selection bias may also be introduced by the use of prevalent rather than incident cases (or a combination of prevalent and incident cases). When a mixture of incident and prevalent cases is used, differences in the genotype distribution between cases and controls might be due to the possible effects of the genotypes on survival rather than on the etiology of the disease of interest (98). Identification of incident cases in SCCHN can be particularly challenging since patients can present with multiple primaries or a second primary after an index diagnosis. Most studies were not clear about whether cases represented the first diagnosis of SCCHN, and at least two acknowledged that a mixture of incident and prevalent cases was included (77, 87).

INTERACTIONS

None of the studies conducted to date have been able to assess gene-environment interaction with precision due to limited statistical power. In addition to adequate sample size, assessment of gene-environment interaction also depends upon the accurate and detailed measurement of exposures and the proper statistical evaluation of interaction on the multiplicative and additive scales.

In general, most case-control studies will require a total sample size of approximately 1,000 persons to achieve 80 percent power when the OR for exposure effect among those without the "at-risk" genotype is 1.5 and the interaction effect is 3.0 or greater (99). The largest studies reviewed consist of fewer than 400 cases and total fewer than 700 persons.

Assessment of gene-environment interaction begins with satisfactory measurement of environmental exposures. Misclassification of exposure, in this case tobacco, can have important effects in gene-environment studies (100). Several studies reviewed either neglected to report history of tobacco use (75, 80, 88, 89) or collapsed tobacco smoking into a binary variable for analysis (72, 73, 78, 83, 90, 93). One study measured tobacco exposure as current/former/never and, thus, measured neither dose nor duration effectively (92).

Measurement of tobacco smoking as a binary variable (such as ever/never) is rarely appropriate, since a broad range of exposure levels will be grouped together by using this strategy. Failure to measure both amount (dose) and length (duration) of lifetime tobacco exposures creates heterogeneity in the assessment of risk. Inaccurate categorization of tobacco exposures may ultimately prevent researchers from identifying genetically susceptible persons who may have increased risk to lower-dose exposures. Additionally, heterogeneous categorization makes comparison across studies difficult.

Among studies that measured dose and duration of tobacco smoking, several adjusted for tobacco in the evaluation of the gene rather than directly assessing the interaction in the analysis (76, 79, 82, 91, 93). Adjustment for the exposure of interest in estimating the main effect of the genotype falls short of the complete assessment of gene-exposure interaction. Full description of a possible gene-exposure interaction requires an epidemiologic and statistical evaluation of interaction (97).

Modest evidence of interaction has been shown with imprecise estimates of effect for risk of SCCHN and *GSTM1*

null genotype among studies that have measured dose and duration of tobacco exposure (85, 94, 95). After adjustment for age and gender, Sato et al. (94) calculated ORs of 3.1 (95 percent CI: 1.6, 5.9), 3.9 (95 percent CI: 1.6, 9.1), and 16.2 (95 percent CI: 4.3, 61.0) for risk of oral cancer for those with the *GSTM1* deletion genotype and increasing lifetime cigarette dose.

Among those studies that have evaluated gene-environment interaction for the *GSTT1* deletion genotype, that by Olshan et al. (85) demonstrated an increasing risk of SCCHN per dose of tobacco smoking after adjustment for age, race, gender, and average number of drinks of alcohol per week. For those with the *GSTT1* deletion genotype who were never smokers, the risk of SCCHN was 2.7 (95 percent CI: 0.5, 12.9) compared with never smokers without the *GSTT1* deletion genotype. Smokers of less than one pack per day had an OR of 3.7 (95 percent CI: 0.7, 19.4), while smokers of one pack per day or more had an OR of 7.0 (95 percent CI: 2.2, 22.0) compared with never smokers without the *GSTT1* deletion genotype.

Finally, a few studies have demonstrated increased risks for patients who have loss of function for combinations of *GSTT1* and *GSTM1*. These studies have demonstrated that persons who are deficient in multiple enzymatic pathways have increased risk for SCCHN (76, 91, 92). Three studies have also suggested an increase in risk for those who have a polymorphism in a phase I enzyme, such as *CYP1A1*, and have the *GSTM1* deletion genotype (77, 94, 84).

Final considerations

Identification of groups of persons who smoke tobacco and may have increased susceptibility for SCCHN based on their ability to metabolize tobacco smoke carcinogens is an important goal. Increased attention needs to be given to methodological considerations such as the appropriate selection of controls, use of incident rather than prevalent cases, and adequate sample size. Measurement of lifetime exposures to tobacco (measured as both dose and duration) will help to minimize heterogeneity in the assessment of gene-environment interaction. Finally, because of the carcinogenic complexity of tobacco smoke and the multistep nature of its metabolism, consideration should be given to including multiple phase I and phase II enzymes as measures of genetic susceptibility.

LABORATORY TESTS

Molecular methods for determining the *GSTM1* and *GSTT1* null genotype have been published previously (26) and will not be reviewed here. All of the studies reviewed extracted genomic DNA from blood samples except for two studies that used exfoliated oral cells only (77, 95) and one that used both blood and oral cells (85). Genotyping methods used in the studies reviewed were consistent with the standard techniques used for PCR (74–76, 78, 79, 81, 83, 86–88, 90, 94), PCR-restriction fragment length polymorphisms (84), and multiplex PCR (77, 80, 82, 85, 89, 91–93, 95). Internal control primers were stated for all studies.

POPULATION TESTING

None as of yet and not indicated.

REFERENCES

1. Hayes J, Pulford D. The glutathione S-transferase supergene family: regulation of GST* and the contribution of the isoenzymes to cancer chemoprevention and drug resistance. Crit Rev Biochem Mol Biol 1995;30:445–600.
2. Zhong S, Howie A, Ketterer B, et al. Glutathione *S*-transferase mu locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. Carcinogenesis 1991;12:1533–7.
3. Warholm M, Rane A, Alexandrie A, et al. Genotypic and phenotypic determination of polymorphic glutathione transferase T1 in a Swedish population. Pharmacogenetics 1995; 5:252–4.
4. Bruhn C, Brockmoller J, Kerb R, et al. Concordance between enzyme activity and genotype of glutathione *S*-transferase theta (*GSTT1*). Biochem Pharmacol 1998;56:1189–93.
5. Rebbeck T. Molecular epidemiology of the human glutathione *S*-transferase genotypes *GSTM1* and *GSTT1* in cancer susceptibility. Cancer Epidemiol Biomarkers Prev 1997; 6:733–43.
6. Hengstler J, Arand M, Herrero ME, et al. Polymorphisms of *N*-acetyltransferases, glutathione *S*-transferases, microsomal epoxide hydrolase and sulfotransferases: influence on cancer susceptibility. Recent Results Cancer Res 1998;154:46–85.
7. Pemble S, Schroeder K, Spencer S, et al. Human glutathione *S*-transferase theta (*GSTT1*): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 1994;300: 271–6.
8. Blot WJ, McLaughlin JK, Winn DM, et al. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 1988;48:3282–7.
9. IARC. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 38. Lyon, France: International Agency for Research on Cancer, 1986.
10. Hecht S. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 1999;91:1194–1210.
11. Vineis P, Malats N, Lang M, et al. Metabolic polymorphisms and susceptibility to cancer. Vol 72, part 2. Lyon, France: International Agency for Research on Cancer, 1999.
12. IARC. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 37. Lyon, France: International Agency for Research on Cancer, 1985.
13. IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Vol 37. Lyon, France: International Agency for Research on Cancer, 1999.
14. Landi S. Mammalian class theta GST and differential susceptibility to carcinogens: a review. Mutat Res 2000;463: 247–83.
15. Pacifici G, Franchi M, Bencini C, et al. Tissue distribution of drug-metabolizing enzymes in humans. Xenobiotica 1988;18: 849–56.
16. Moscow J, Fairchild C, Madden M, et al. Expression of anionic glutathione *S*-transferase and *P*-glycoprotein genes in human tissues and tumors. Cancer Res 1989;49:1422–8.
17. Howie A, Forrester L, Glancey M, et al. Glutathione *S*-transferase and glutathione peroxidase expression in normal and tumour human tissues. Carcinogenesis 1990;3:451–8.
18. Janot F, Massaad L, Ribrag V, et al. Principal xenobiotic-metabolizing enzyme systems in human head and neck squamous cell carcinoma. Carcinogenesis 1993;14:1279–83.
19. Bongers V, Snow GB, Braakhuis BJ. The role of glutathione *S*-transferases in head and neck squamous cell carcinogenesis. Eur J Cancer B Oral Oncol 1995;31B:349–54.
20. Mulder T, Manni J, Roelofs H, et al. Glutathione *S*-transferases

and glutathione in human head and neck cancer. Carcinogenesis 1995;16:619–24.
21. Mulder T, Manni J, Roelofs H, et al. Glutathione peroxidases in human head and neck cancer. Acta Otolaryngol 1995; 115:331–3.
22. Gaffey MJ, Iezzoni JC, Meredith SD, et al. Cyclin D1 (PRAD1, CCND1) and glutathione *S*-transferase pi gene expression in head and neck squamous cell carcinoma. Hum Pathol 1995;26:1221–6.
23. Chen YK, Lin LM. Evaluation of glutathione *S*-transferase activity in human buccal epithelial dysplasias and squamous cell carcinomas. Int J Oral Maxillofac Surg 1997;26:205–9.
24. Ulrich D, Munzel P, Beck-Gschaidmeier S, et al. Drug-metabolizing enzymes in pharyngeal mucosa and in oropharyngeal cancer tissue. Biochem Pharmacol 1997;54:1159–62.
25. Ophuis M, Mulder T, Peters W, et al. Plasma glutathione *S*-transferase P1-1 levels in patients with head and neck squamous cell carcinoma. Cancer 1998;82:2434–8.
26. Cotton S, Sharp L, Little J, et al. Glutathione *S*-transferase polymorphisms and colorectal cancer: HuGE review. Am J Epidemiol 2000;151:7–32.
27. Rebbeck T, Walker A, Jaffe J, et al. Glutathione *S*-transferase-mu (*GSTM1*) abd -theta (*GSTT1*) genotypes in the etiology of prostate cancer. Cancer Epidemiol Biomarkers Prev 1999;8: 283–7.
28. Chen C, Madeleine M, Weiss N, et al. Glutathione *S*-transferase M1 genotypes and the risk of squamous carcinoma of the cervix: a population-based case-control study. Am J Epidemiol 1999;150:568–72.
29. Chen C, Madeleine MM, Weiss NS, et al. Glutathione *S*-transferase M1 genotypes and the risk of vulvar cancer: a population-based case-control study. Am J Epidemiol 1999; 150:437–42.
30. Gertig D, Stampfer M, Haiman C, et al. Glutathione *S*-transferase *GSTM1* and *GSTT1* polymorphisms and colorectal cancer risk: a prospective study. Cancer Epidemiol Biomarkers Prev 1998;7:1001–5.
31. Crump C, Chen C, Appelbaum F, et al. Glutathione *S*-transferase theta 1 gene deletion and risk of acute myeloid leukemia. Cancer Epidemiol Biomarkers Prev 2000;9: 457–60.
32. Quinones L, Berthou F, Varela N, et al. Ethnic susceptibility to lung cancer: differences in *CYP2E1*, *CYP1A1*, and *GSTM1* genetic polymorphisms between French Caucasian and Chilean populations. Cancer Lett 1999;141:167–71.
33. Arruda V, Grignolli C, Goncalves M, et al. Prevalence of homozygosity for the deleted alleles of glutathione *S*-transferase mu (*GSTM1*) and theta (*GSTT1*) among distinct ethnic groups from Brazil: relevance to environmental carcinogenesis? Clin Genet 1998;54:210–14.
34. Stucker I, de Waziers I, Cenee S, et al. *GSTM1*, smoking and lung cancer: a case-control study. Int J Epidemiol 1999;28: 829–35.
35. Palli D, Vineis P, Russo A, et al. Diet, metabolic polymorphisms and DNA adducts: the EPIC-Italy cross-sectional study. Int J Cancer 2000;87:444–51.
36. Kiss I, Sandor J, Pajkos G, et al. Colorectal cancer risk in relation to genetic polymorphism of cytochrome P450 1A1, 2E1, and glutathione-*S*-transferase M1 enzymes. Anticancer Res 2000;20:519–22.
37. Salagovic J, Kalina I, Habalova V, et al. The role of human glutathione *S*-transferases M1 and T1 in individual susceptibility to bladder cancer. Physiol Res 1999;48:465–71.
38. Setiwan V, Zhang Z, Yu G, et al. *GSTT1* and *GSTM1* null genotypes and the risk of gastric cancer: a case-control study in a Chinese population. Cancer Epidemiol Biomarkers Prev 2000;9:73–80.
39. Park SK, Yoo KY, Lee SJ, et al. Alcohol consumption, glutathione *S*-transferase M1 and T1 genetic polymorphisms and breast cancer risk. Pharmacogenetics 2000;10:301–9.
40. Kim WJ, Lee HL, Lee SC, et al. Polymorphisms of *N*-acetyltransferase 2, glutathione *S*-transferase mu and theta genes as risk factors of bladder cancer in relation to asthma and tuberculosis. J Urol 2000;164:209–13.
41. Lee EJ, Wong JY, Yeoh PN, et al. Glutathione *S*-transferase theta (*GSTT1*) genetic polymorphism among Chinese, Malays and Indians in Singapore. Pharmacogenetics 1995;5: 332–334.
42. Landis SH, Murray T, Bolden S, et al. Cancer statistics, 1999. CA Cancer J Clin 1999;49:8–31.
43. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999;80: 827–41.
44. La Vecchia C, Tavani A, Franceschi S, et al. Epidemiology and prevention of oral cancer. Oral Oncol 1997;33:302–12.
45. Blot W, McLaughlin J, Devesa S, et al. Cancers of the oral cavity and pharynx. In: Schottenfeld D, Fraumeni JF Jr, eds. Cancer epidemiology and prevention: London, England: Oxford University Press, 1996:666–80.
46. Ildstad S, Tollerud D, Bigelow ME. Squamous cell carcinoma of the head and neck at the Massachusetts General Hospital: a comparison of biologic characteristics in men and women. Surgery 1986;99:7–13.
47. Kabat GC, Hebert JR, Wynder EL. Risk factors for oral cancer in women. Cancer Res 1989;49:2803–6.
48. Muscat JE, Richie JP Jr, Thompson S, et al. Gender differences in smoking and risk for oral cancer. Cancer Res 1996;56: 5192–7.
49. Winn DM, Blot WJ, Shy CM, et al. Snuff dipping and oral cancer among women in the southern United States. N Engl J Med 198;304:745–9.
50. Winn DM, Blot WJ, Shy CM, et al. Occupation and oral cancer among women in the South. Am J Ind Med 1982;3:161–7.
51. Winn DM, Ziegler RG, Pickle LW, et al. Diet in the etiology of oral and pharyngeal cancer among women from the southern United States. Cancer Res 1984;44:1216–22.
52. Winn DM, Pickle LW. Smokeless tobacco and cancer in women: implications for cancer research. Women Health 1986;11:253–66.
53. Schlecht N, Franco E, Pintos J, et al. Interaction between tobacco and alcohol consumption and the risk of cancers of the upper aero-digestive tract in Brazil. Am J Epidemiol 1999;150:1129–37.
54. Gillison M, Koch W, Shah K. Human papillomavirus in head and neck squamous cell carcinoma: are some head and neck cancers a sexually transmitted disease? Curr Opin Oncol 1999;11:191–9.
55. Maden C, Beckman AM, Thomas DB, et al. Human papillomaviruses, herpes simplex viruses, and the risk of oral cancer in men. Am J Epidemiol 1992;135:1093–1102.
56. Smith E, Hoffman H, Summersgill K, et al. Human papillomavirus and risk of oral cancer. Laryngoscope 1998;108: 1098–1103.
57. Gillison M, Koch W, Capone R, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000;92:709–20.
58. McKaig R, Baric R, Olshan A. Human papillomavirus and head and neck cancer: epidemiology and molecular biology. Head Neck 1998;20:250–65.
59. Boffetta P, Merletti F, Magnani C, et al. A population-based study of prognostic factors in oral and oropharyngeal cancer. Eur J Cancer B Oral Oncol 1994;30B:369–73.
60. Koch WM, Patel H, Brennan J, et al. Squamous cell carcinoma of the head and neck in the elderly. Arch Otolaryngol Head Neck Surg 1995;121:262–5.
61. Day GL, Blot WJ, Austin DF, et al. Racial differences in risk of oral and pharyngeal cancer: alcohol, tobacco, and other determinants. J Natl Cancer Inst 1993;85:465–73.
62. Gridley G, McLaughlin JK, Block G, et al. Diet and oral and pharyngeal cancer among blacks. Nutr Cancer 1990;14: 219–25.
63. Greenberg RS, Haber MJ, Clark WS, et al. The relation of socioeconomic status to oral and pharyngeal cancer. Epidemiology 1991;2:194–200.
64. Merletti F, Boffetta P, Ferro G, et al. Occupation and cancer

of the oral cavity or oropharynx in Turin, Italy. Scand J Work Environ Health 1991;17:248–54.
65. Macfarlane GJ, Zheng T, Marshall JR, et al. Alcohol, tobacco, diet and the risk of oral cancer: a pooled analysis of three case-control studies. Eur J Cancer B Oral Oncol 1995; 31B:181–7.
66. Marshall J, Boyle P. Nutrition and oral cancer. Cancer Causes Control 1996;7:101–11.
67. Winn D. Diet and nutrition in the etiology of oral cancer. Am J Clin Nutr 1995;61:437S–45S.
68. Glade M. Food, nutrition, and the prevention of cancer: a global perspective. American Institute for Cancer Research/World Cancer Research Fund, American Institute for Cancer Research, 1997. Nutrition 1999;15:523–6.
69. IARC. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. (Vol 36). Lyon, France: International Agency for Research on Cancer, 1985.
70. Blot WJ, Winn DM, Fraumeni JF Jr. Oral cancer and mouthwash. J Natl Cancer Inst 1983;70:251–3.
71. Winn DM. Epidemiology of cancer and other systemic effects associated with the use of smokeless tobacco. Adv Dental Res 1997;11:313–21.
72. LaFuente A, Pujol F, Carretero P, et al. Human glutathione *S*-transferase mu deficiency as a marker for the susceptibility to bladder and larynx cancer among smokers. Cancer Lett 1993; 68:49–54.
73. LaFuente A, Maristany M, Arias C, et al. Glutathione and glutathione *S*-transferases in human squamous cell carcinomas of the larynx and *GSTM1* dependent risk. Anticancer Res 1998;18:107–12.
74. Coutelle C, Ward PJ, Fleury B, et al. Laryngeal and oropharyngeal cancer, and alcohol dehydrogenase 3 and glutathione *S*-transferase M1 polymorphisms. Hum Genet 1997;99: 319–25.
75. Deakin M, Elder J, Hendrickse C, et al. Glutathione *S*-transferase *GSTT1* genotypes and susceptibility to cancer: studies of interactions with *GSTM1* in lung, oral, gastric, and colorectal cancers. Carcinogenesis 1996;17:881–4.
76. Hung H, Chuang J, Chien Y, et al. Genetic polymorphisms of *CYP2E1*, *GSTM1*, and *GSTT1*; environmental factors and risk of oral cancer. Cancer Epidemiol Biomarkers Prev 1997; 6:901–5.
77. Park J, Muscat J, Ren Q, et al. *CYP1A1* and *GSTM1* polymorphisms and oral cancer risk. Cancer Epidemiol Biomarkers Prev 1997;6:791–7.
78. Gonzalez M, Alvarez V, Pello M, et al. Genetic polymorphism of *N*-acetyltransferase-2, glutathione *S*-transferase-M1, and cytochromes p450 IIE1 and p450 IID6 in the susceptibility to head and neck cancer. J Clin Pathol 1998; 51:294–8.
79. Matthias C, Bockmuhl U, Jahnke V, et al. Polymorphism in cytochrome P450 *CYP2D6, CYP1A1, CYP2E1,* and glutathione *S*-transferase, *GSTM1, GSTM3, GSTT1* and susceptibility to tobacco-related cancers: studies in upper aerodigestive tract cancers. Pharmacogenetics 1998;8:91–100.
80. Ophuis M, van Lieshout E, Roelofs H, et al. Glutathione *S*-transferase M1 and T1 and cytochrome P4501A1 polymorphisms in relation to the risk for benign and malignant head and neck lesions. Cancer 1998;82:936–43.
81. Worrall S, Corrigan M, High A, et al. Susceptibility and outcome in oral cancer: preliminary data showing an association with polymorphism in cytochrome P450 *CYP2D6*. Pharmacogenetics 1998;8:433–9.
82. Jourenkova-Mironova N, Voho A, Bouchardy C, et al. Glutathione *S*-transferase *GSTM1*, *GSTM3*, *GSTP1* and *GSTT1* genotypes and the risk of smoking-related oral and pharyngeal cancers. Int J Cancer 1999;81:44–8.
83. Morita S, Yano M, Tsujinnaka T, et al. Genetic polymorphisms of drug-metabolizing enzymes and susceptibility to head-and-neck squamous-cell carcinoma. Int J Cancer 1999; 80:685–8.
84. Tanimoto K, Hayashi S, Yoshiga K, et al. Polymorphisms of the *CYP1A1* and *GSTM1* gene involved in oral squamous cell carcinoma in association with a cigarette dose. Oral Oncol 1999;35:191–6.
85. Olshan A, Weissler M, Watson M, et al. *GSTM1*, *GSTT1*, *GSTP1*, *CYP1A1*, and *NAT1* polymorphisms, tobacco use, and the risk of head and neck cancer. Cancer Epidemiol Biomarkers Prev 2000;9:185–91.
86. McWilliams J, Evans A, Beer T, et al. Genetic polymorphisms in head and neck cancer risk. Head Neck 2000;22: 609–17.
87. Hamel N, Karimi S, Hebert-Blouin M, et al. Increased risk of head and neck cancer in association with *GSTT1* nullizygosity for individuals with low exposure to tobacco. Int J Cancer 2000;87:452–4.
88. Jahnke V, Matthias C, Fryer A, et al. Glutathione *S*-transferase and cytochrome-P-450 polymorphism as risk factors for squamous cell carcinoma of the larynx. Am J Surg 1996;172: 671–3.
89. Trizna Z, Clayman GL, Spitz MR, et al. Glutathione *S*-transferase genotypes as risk factors for head and neck cancer. Am J Surg 1995;170:499–501.
90. Kihara M, Kihara M, Kubota A, et al. *GSTM1* gene polymorphism as a possible marker for susceptibility to head and neck cancers among Japanese smokers. Cancer Lett 1997;112: 257–62.
91. Jourenkova N, Reinikainen M, Bouchardy C, et al. Larynx cancer risk in relation to glutathione *S*-transferase M1 and T1 genotypes and tobacco smoking. Cancer Epidemiol Biomarkers Prev 1998;7:19–23.
92. Cheng L, Sturgis E, Eicher S, et al. Glutathione *S*-transferase polymorphisms and risk of squamous cell carcinoma of the head and neck. Int J Cancer 1999;84:220–4.
93. Katoh T, Kaneko S, Kohshi K, et al. Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and oral cavity cancer. Int J Cancer 1999;83:606–9.
94. Sato M, Sato T, Izumo T, et al. Genetic polymorphism of drug-metabolizing enzymes and susceptibility to oral cancer. Carcinogenesis 1999;20:1927–31.
95. Park JL, Muscat JE, Kaur T, et al. Comparison of *GSTM* polymorphisms and risk for oral cancer between African-Americans and Caucasians. Pharmacogenetics 2000;10: 123–31.
96. Jahnke V, Strange R, Matthias C, et al. Glutathione *S*-transferase and cytochrome P450 genotypes as risk factors for laryngeal carcinoma. Eur Arch Otorhinolaryngol Suppl 1997;1:S147–9.
97. Rothman K, Greenland S. Modern epidemiology. 2nd ed. Philadelphia, PA: Lippincott-Raven, 1998.
98. Khoury M, Beaty T, Cohen B. Fundamentals of genetic epidemiology. New York, NY: Oxford University Press, 1993.
99. Garcia-Closas M, Lubin JH. Power and sample size calculations in case-control studies of gene-environment interactions: comments on different approaches. Am J Epidemiol 1999;149:689–92.
100. Garcia-Closas M, Rothman N, Lubin J. Misclassification in case-control studies of gene-environment interactions: assessment of bias and sample size. Cancer Epidemiol Biomarkers Prev 1999;8:1043–50.

APPENDIX. INTERNET WEBSITES

National Cancer Institute
http:/www.nci.nih.gov
HuGE
http:/www.cdc.gov/genetics/hugenet